Microsomal Epoxide Hydrolase Polymorphisms and Risk for Advanced Colorectal Adenoma

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Abstract

Cigarette smoking is a risk factor for colorectal adenoma, a precursor of colorectal cancer. Microsomal epoxide hydrolase (EPHX1) metabolizes polycyclic aromatic hydrocarbons, carcinogens found in cigarette smoke. Nonsynonymous variants of EPHX1 at $Tyr_{113}His$ (exon 3) and $His_{139}Arg$ (exon 4) are associated, respectively, with low ($_{113}$His) and high ($_{139}$Arg) predicted activity. Among participants randomized to the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, we evaluated risks for advanced adenoma in relation to cigarette use and these two EPHX1 variants. We compared 772 cases with advanced adenoma (adenoma ≥1 cm or containing high-grade dysplasia or villous, including tubulovillous, elements) of the distal colon (left-sided, descending colon and sigmoid or rectum) to 777 gender- and age-matched controls who were screen-negative for left-sided adenoma. Compared to those with homozygous genotypes predicting low EPHX1 activity, advanced adenoma risks tended to be elevated for carriers of $_{113}$TyrTyr [odds ratios (OR), 1.5; 95% confidence intervals (CI), 1.0-2.2] and $_{139}$ArgArg (OR, 1.4; 95% CI, 0.8-2.5) and for subjects who carried a greater number of the alleles ($_{113}$Tyr or $_{139}$Arg) associated with high predicted enzymatic activity (P_{trend} = 0.03). The increased risk associated with the increasing number of putative high-activity alleles was most apparent among current and recent (quit <10 years) cigarette smokers (P_{trend} = 0.02). In conclusion, EPHX1 variants at codon 113 and 139 associated with high predicted enzymatic activity appear to increase risk for colorectal adenoma, particularly among recent and current smokers. (Cancer Epidemiol Biomarkers Prev 2005;14(1):152–57)

Introduction

Colorectal carcinogenesis involves both environmental and genetic factors (1, 2). Colorectal adenomas are cancer precursors (3). Identifying the determinants of these premalignant lesions is important for understanding the causes of colorectal cancer. We (4) and others (5) have related cigarette use, and particularly current and recent use, to increased risk for colorectal adenoma.

Microsomal epoxide hydrolase (EPHX1) metabolizes a broad array of epoxide substrates, including polycyclic aromatic hydrocarbons (PAH), carcinogens found in cigarette smoke (6). EPHX1 converts the tobacco combustion product benzo(*a*)pyrene-derived benzo(*a*)pyrene 7,8-epoxide to the less toxic transdihydrodiol derivative, benzo(*a*)pyrene 7,8 diol (7). Although the enzyme activity of EPHX1 is detoxifying with respect to the epoxide, the diol subsequently serves as the primary substrate for cytochrome *P*450 conversion to the highly reactive benzo(*a*)pyrene 7,8 dihydrodiol 9,10-epoxide (BPDE, ref. 7). BPDE forms adducts in DNA hotspots and is considered a major tobacco-derived benzo(*a*) pyrene carcinogen (8). PAH are also found in foods cooked at high temperatures, although typically resulting in lower doses than that derived from tobacco smoke (9).

The several-fold variation in substrate-specific activity of EPHX1 in humans is partly attributable to nonsynonymous genetic polymorphisms (that result in amino acid substitutions) in exon 3 ($Tyr_{113}His$, rs1051740) and exon 4 ($His_{139}Arg$, rs2234922; ref. 10). *In vitro*, EPHX1 activity is reduced (about 40%) associated with $_{113}$His and increased (about 25%) associated with $_{139}$Arg, probably due to altered protein stability (11). Furthermore, predicted high epoxide hydrolase activity, based on combined EPHX1 genotypes at codons 113 and 139, is associated with increased BPDE DNA adducts (12) and chromosomal aberrations (13).

Because of discrepant findings in earlier epidemiologic studies (7, 10, 14-16) of generally smaller sample sizes, we investigated advanced colorectal adenoma risk in relation to the EPHX1 nonsynonymous polymorphisms at codon 113 and 139 among participants in a large screening trial for the early detection of colorectal cancer. As our studies had previously shown that tobacco-related risk for adenoma was largely limited to recent (including current) and heavy smokers (4), we hypothesized that EPHX1-associated risks should be evident primarily in these groups.

Materials and Methods

The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The National Cancer Institute's Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial randomized 77,483 screening arm participants (38,364 men, 39,119 women) and a similar number of nonscreened controls, ages 55 to 74, at 10 US screening centers (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake City, UT,

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St. Louis, MO, and Washington, DC; ref. 17). Eligible subjects reported no prior personal history of prostate, lung, colorectal or ovarian cancer. Criteria for exclusion included (*a*) current treatment for cancer (excluding basal cell and squamous cell skin cancer), (*b*) prior total colectomy, pneumonectomy, prostatectomy, or bilateral oophorectomy (with bilateral oophorectomy dropped as an exclusion criterion beginning in 1996), (*c*) participation in another cancer screening or primary prevention study, and (*d*) recent usage of finasteride (Proscar) or tamoxifen (Nolvadex). Beginning in April 1995, PLCO excluded men reporting more than one prostate-specific antigen blood test, and men and women reporting any lower gastrointestinal procedure (proctoscopy, sigmoidoscopy, barium enema, or colonoscopy) within 3 years before study enrollment. The primary method for recruiting study subjects involved mailing informational brochures and letters of invitation to age-eligible persons identified on public, commercial, or screening center–specific mailing lists.

Physician and nonphysician examiners, all centrally registered, followed standardized procedures to perform and record results from an initial 60-cm flexible sigmoidoscopy examination, done as soon as possible after study entry. As a general rule, PLCO examiners, at the time of the screening examination, did not biopsy or remove polyps or masses. Rather, PLCO referred subjects with screen-detected abnormalities to personal physicians for diagnostic follow-up. PLCO abstracted medical records pertaining to subsequent diagnostic work-ups. Information abstracted from medical records included the occurrence and date of follow-up flexible sigmoidoscopy and/or colonoscopy examinations, the anatomic location, size (visual estimate as recorded on clinical endoscopy reports), histology of polyps and masses observed on follow-up, dates of diagnosis, and tumor-node-metastasis clinical and pathologic stages for subjects with invasive colorectal cancers. Questionnaire data and biological samples were acquired from study participants (18). Participants provided written informed consent. The study was approved by the institutional review boards of the National Cancer Institute and the 10 screening centers.

Study Population. Cases and controls for this study of EPHX1, tobacco use, and adenoma risk were drawn from screening-arm participants at the 10 screening centers of the PLCO Trial who filled out risk factor questionnaires had a successful sigmoidoscopy (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified), and provided a blood sample for use in etiologic studies (Sep. 93-Sep. 99 applied all the conditions described), (n = 42,037; ref. 19). Of these participants, we excluded 4,834 with a self-reported history of ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, Gardner's syndrome, or cancer (except basal cell and squamons cell skin cancer). We randomly selected 772 of 1,234 cases with at least one advanced colorectal adenoma (adenoma ≥1 cm or containing high-grade dysplasia or villous, including tubulovillous, elements) in the distal colon (descending colon and sigmoid or rectum), and 777 of 26,651 control participants, with a negative sigmoidoscopy screening (i.e., no polyp or other suspect lesion), frequency-matched to the cases by gender and ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, and others). Study subjects were predominantly non-Hispanic Whites (94%). Among the 772 cases, 572 (74%) had a lesion ≥1 cm, 489 (63%) showed advanced histologic features, and 245 (32%) had multiple adenoma. Also, 631 (82%) cases had an advanced adenoma of the descending colon or sigmoid and 232 (30%) had an advanced adenoma of the rectum, including subjects having lesions at both sites.

Genotype Assay. DNA samples were obtained from stored blood samples using Qiagen standard protocols (QIAamp DNA Blood Midi or Maxi kit: http://www1.qiagen.com/default.aspx). Genotyping was conducted by TaqMan (Applied Biosystems, Foster City, CA). All assays were validated and optimized at the NCI Core Genotyping Facility Laboratory http://snp500cancer.nci.nih.gov). Internal laboratory quality controls were Coriell DNA samples containing homozygous major allele, heterozygous, and homozygous minor allele genotypes for each polymorphism under study with four of each control type and four no template controls in every 384 samples. For external blinded quality controls, we interspersed ~10% repeated quality control samples from 40 individuals. The blinded samples showed >99% interassay concordance for both assays. Genotype data were successfully obtained for 92% to 93% of study subjects excluding individuals with insufficient DNA (1%), genotyping failures (5-6%), and fingerprint profile review showing subject-specific ambiguities (1%).

Questionnaire Data. Participants completed a baseline general risk factor questionnaire and a 137-item food frequency questionnaire, to report the use of tobacco, alcohol, selected drugs and hormones, body size, and usual dietary intake over the 12 months prior to enrollment. Detailed information on smoking history was collected, including ages started and stopped, total years of use, amount usually used, and type of tobacco used (cigarettes, pipes, and cigars). Subjects who did not smoke cigarettes for more than 6 months or did not smoke pipes or cigars for more than a year were considered to be nonsmokers. For evaluation of risks in relation to time period of cigarette use, cigarette users were classified as long-term quitters (quit ≥10 years before enrollment) and current or recent smokers (quit <10 years before enrollment). Dietary nutrient intake was calculated by multiplying the reported frequency of consumption for relevant food items by gender- and nutrient-specific portion sizes (20) using the nutrient database from the U.S. Department of Agriculture (21). Dietary intake of benzo(*a*)pyrene was calculated using a database developed by Kazerouni et al. (22).

Statistical Analysis. Similar to previous studies (7, 23, 24), we inferred that alleles associated with more stable protein sequences (i.e., $_{113}$Tyr and $_{139}$Arg) predicted higher total activity of EPHX1. A combined genotype score was derived from the codon 113 and 139 polymorphisms of EPHX1 by calculating the total number of putative high-activity alleles ($_{113}$Tyr and $_{139}$Arg). Subjects were characterized as having low, medium, or high predicted activities, respectively, depending on whether they carried no or one, two, or three to four copies of the putative high-activity alleles.

Departure from Hardy-Weinberg equilibrium was assessed by comparing the expected to observed genotype frequencies using the asymptotic Pearson's χ^2 test. Odds ratios (OR) and 95% confidence intervals (CI) were obtained using unconditional logistic regression, adjusting for gender, race (non-Hispanic White, non-Hispanic Black, and others), and age (55-59, 60-64, 65-69, and 70-74). We did trend tests for the three-level combined genotype variable using logistic regression models based on the integer score (0, 1, 2). Statistical significance of multiplicative interaction between EPHX1 genotype and smoking was tested using the Wald test for the interaction term in the logistic regression models.

Based on a novel extension of polytomous logistic regression for multivariate outcome analysis (25), we studied whether the effect of EPHX1 alleles varied for three characteristics of adenomas: size (≥1 versus <1 cm), multiplicity (multiple versus single), and advanced histologic features (high-grade dysplasia or villous versus absence of these features), estimating case to case ORs for each characteristic after controlling for the other two characteristics. Adenoma risks associated with haplotypes defined by multiple polymorphisms were assessed by the SNPEM program (26, 27), using

an expectation-maximization algorithm to estimate haplotype frequencies and permutation tests to evaluate differences between cases and controls. All *P* values were two-sided. Individuals with missing values were excluded from specific analyses.

Results

Distributions of the matching factors of gender and race were similar for cases and controls, however, cases tended to have a higher body mass index, and were older, more likely to have reported a first-degree family history of colorectal cancer, and have had less education (Table 1). Cigarette smoking was associated with increased risk for advanced adenoma (OR, 1.4; 95% CI, 1.2-1.8; Table 2), with greater risks among current and recent cigarette smokers (OR, 2.4; 95% CI, 1.8-3.2). Risks by usual amount smoked were OR, 1.3 (95% CI, 1.0-1.6) for ≤20 cigarettes per day and OR, 1.7 (95% CI, 1.3-2.2) for >20 cigarettes per day.

Among control subjects, minor allele frequencies for the EPHX1 variants were 31% for $_{113}$His (low) and 19% for $_{139}$Arg (high; Table 3). Both polymorphisms were in Hardy-Weinberg equilibrium (P = 0.1 for EPHX1$_{113}$ and 0.8 for EPHX1$_{139}$). Risks for advanced adenoma tended to be elevated among subjects who carried one or two copies of EPHX1$_{113}$Tyr (OR, 1.5; 95% CI, 1.0-2.2; Table 3), among subjects homozygous for EPHX1$_{139}$Arg (OR, 1.4; 95% CI, 0.8-2.5), and among subjects who carried a greater number of alleles ($_{113}$Tyr or $_{139}$Arg) associated with high predicted enzymatic activity (P_{trend} = 0.03). Risks associated with the EPHX1 genotypes were similar for men and women (data not shown). In multivariate outcome analyses, we found differential risks associated with high predicted EPHX1 activity for subjects with multiple versus single adenoma (case to case OR for two high-activity alleles, 1.6; 95% CI, 1.0-2.4; case to case OR for three to four high-activity alleles, 1.5; 95% CI, 0.9-2.3). No differences in genotype effect were observed for adenoma size or histology. Risks were similar for subjects with advanced adenoma of the descending colon and sigmoid versus the rectum, using standard logistic regression analysis.

Excess risks associated with the high predicted EPHX1 enzymatic activity were apparent only among current and recent smokers (OR for three to four versus zero to one high-activity alleles, 2.2; 95% CI, 1.2-4.2; P_{trend} = 0.02; Table 4). In analyses restricted to current smokers only, similar associations were found with wider confidence intervals (data not shown). Risks were greatest for current and recent heavy smokers (>20 cigarettes/day; OR for three to four versus zero

Table 1. Selected characteristics of subjects in a nested case-control study of advanced colorectal adenoma in the PLCO Cancer Screening Trial

	Case, n = 772*		Control, n = 777*		P_{χ^2}
	n	(%)	n	(%)	
Matching factors					
Gender					
Male	535	(69.3)	536	(69.0)	0.9
Female	237	(30.7)	241	(31.0)	
Race					
White	725	(93.9)	729	(93.8)	1.0
Black	22	(2.9)	23	(3.0)	
Other†	25	(3.2)	25	(3.2)	
Other factors					
Age (years)					
55-59	257	(33.3)	363	(46.7)	<0.0001
60-64	244	(31.6)	200	(25.7)	
65-69	172	(22.3)	140	(18.0)	
70-74	99	(12.8)	74	(9.5)	
First-degree family history of colorectal cancer					
Yes	97	(12.6)	70	(9.0)	0.02
No	675	(87.4)	707	(91.0)	
Education					
<12 years	72	(9.3)	50	(6.4)	0.001
12 years/high school equivalent	191	(24.7)	176	(22.7)	
Some college	276	(35.8)	247	(31.8)	
College and above	232	(30.1)	303	(39.0)	
Body mass index at interview					
<18.5	5	(0.7)	2	(0.3)	0.3
≥18.5-<25	200	(25.9)	219	(28.2)	
≥25-<30	349	(45.2)	357	(46.0)	
≥30	215	(27.9)	188	(24.2)	
Center					
Colorado	71	(9.2)	89	(11.5)	<0.0001
Georgetown	36	(4.7)	45	(5.8)	
Hawaii	15	(1.9)	14	(1.8)	
Henry Ford Health System	78	(10.1)	107	(13.8)	
Minnesota	136	(17.6)	174	(22.4)	
Washington	77	(10.0)	75	(9.7)	
Pittsburgh	124	(16.1)	59	(7.6)	
Utah	69	(8.9)	41	(5.3)	
Marshfield	135	(17.5)	156	(20.1)	
Alabama	31	(4.0)	17	(2.2)	

*Numbers do not add up to the total because of missing values.
†Hispanic (0.9%), Asian (1.7%), Pacific islander (0.4%), and American Indian native (0.3%).

Table 2. Risks of advanced colorectal adenoma associated with tobacco use in the PLCO Cancer Screening Trial

	Case (%), n = 772*	Control (%), n = 777*	OR (95% CI)†
Tobacco use			
No tobacco	260 (33.7)	315 (40.5)	1.0
No cigarettes, but pipes or cigars	39 (5.1)	43 (5.5)	1.2 (0.7-1.9)
Cigarette smoker	473 (61.3)	419 (53.9)	1.4 (1.2-1.8)
Long-term quitters (quit ≥10 years)	272 (35.2)	302 (38.9)	1.1 (0.9-1.4)
Current or recent (quit <10 years)	198 (25.6)	111 (14.3)	2.4 (1.8-3.2)
Light (≤20 cigarettes/d)	261 (35.6)	254 (34.6)	1.3 (1.0-1.6)
Heavy (>20 cigarettes/d)	212 (28.9)	165 (22.5)	1.7 (1.3-2.2)

*Numbers do not add up to the total because of missing values.
†Adjusted for gender, race, and age.

to one high-activity alleles, 3.3; 95% CI, 1.3-8.7; P_{trend} = 0.02). No significant pattern of genotype effect was observed for smokers who quit ≥10 years before enrollment (P_{trend} =1.0) or for nonsmokers (P_{trend} = 0.2). Formal tests of interaction revealed a borderline-significant risk modification ($P_{interaction}$ = 0.06) between the EPHX1 combined genotype (three to four versus zero to two high-activity alleles) and smoking status (current and recent smokers versus long-term quitters and nonsmokers). Also in current and recent smokers, the $_{113}$Tyr-$_{139}$Arg haplotype (11% of controls, 19% of cases), compared with all other haplotypes, was associated with increased risk (OR, 1.9, P value = 0.01), whereas the haplotypes containing only one high-activity allele ($_{113}$Tyr-$_{139}$His or $_{113}$His-$_{139}$Arg) and the $_{113}$His-$_{139}$His haplotype were not associated with risk (OR, 0.8, 0.9, 0.9, respectively). A similar but weaker interaction pattern was seen for theinteraction of cigarette use (>20 cigarettes per day versus others) and genotype (three to four versus zero to two high–activity alleles) with adenoma risk ($P_{interaction}$ = 0.6).

The risks associated with EPHX1 genotypes were not modified by dietary red meat or estimated dietary benzo(*a*) pyrene intake, when considering all subjects or when restricted to nonsmokers ($P_{interaction}$ = 0.6-0.9 for the combined genotype of EPHX1$_{113}$ and EPHX1$_{139}$). Adjustment for other suspected confounders (e.g., screening center, first-degree family history of colorectal cancer, education, body mass index, dietary intake of fiber and red meat, and postmenopausal hormone use among women) did not substantively change the relationships observed between genotype, smoking, and risk for advanced adenoma. When analyses were restricted to non-Hispanic Whites, all results were essentially the same.

Discussion

In our study of 772 cases and 777 controls, we found that risks for advanced colorectal adenoma tended to be greatest among carriers of two nonsynonymous variants in EPHX1 ($_{113}$Tyr and $_{139}$Arg) related to high predicted enzymatic activity, particularly among current and recent smokers. Haplotype analysis showed the strongest risks associated with carriage of two at-risk alleles on the same chromosome, pointing to potential *cis* effects of the two variants.

Our findings are in close agreement with reports by Cortessis et al. (7) of 464 sigmoidoscopy-identified adenoma cases and 510 matched controls, showing a >4-fold increase in adenoma risk in current smokers with high predicted activity of EPHX1, and by Tiemersma et al. (15) of 385 endoscopy-identified adenoma cases and 396 polyp-free controls, showing a 2-fold risk among current smokers carrying the EPHX1 putative high-activity alleles. Sachse et al. (16), in 490 colorectal cancer patients and 593 controls, found comparable increased risks for colorectal cancer associated with the EPHX1$_{113}$Tyr high-activity variant, but did not take tobacco use into account. In contrast, a study by Ulrich et al. (10), in 530 adenoma and 649 polyp-free controls at colonoscopy, found a protective effect associated with the putative high–activity EPHX1 genotypes among heavy smokers. Also, Harrison et al. (14) in 101 colon cancer patients and 203 controls, found reduced risks associated with the EPHX1$_{113}$ residue predicting high enzymatic activity and no effect associated with the EPHX1$_{139}$ variant.

Reasons for the discrepancy in results are unclear. Neither study of cancer (14, 16) considered interrelationships with tobacco use. Ours was the only study to report specifically on

Table 3. Risks of advanced colorectal adenoma associated with EPHX1 genotypes in the PLCO Cancer Screening Trial

	Case (%), n = 772*	Control (%), n = 777*	Minor allele frequency in controls	OR (95% CI)†
EPHX1 $_{113}$Tyr allele				
0 (low)	56 (7.9)	80 (11.0)	0.31	1.0
1 (medium)	299 (42.0)	291 (39.9)		1.5 (1.0-2.2)
2 (high)	357 (50.1)	358 (49.1)		1.5 (1.0-2.2)
P_{trend}				0.2
EPHX1 $_{139}$Arg allele				
0 (low)	443 (63.1)	479 (66.2)	0.19	1.0
1 (medium)	228 (32.5)	221 (30.5)		1.1 (0.9-1.4)
2 (high)	31 (4.4)	24 (3.3)		1.4 (0.8-2.5)
P_{trend}				0.2
Combined number of $_{113}$Tyr or $_{139}$Arg alleles				
0-1 (low)	245 (31.7)	263 (33.9)	—	1.0
2 (medium)	285 (36.9)	317 (40.8)		1.0 (0.8-1.8)
3-4 (high)	242 (31.4)	197 (25.4)		1.4 (1.0-1.8)
P_{trend}				0.03

*Numbers do not add up to the total because of missing values.
† Adjusted for gender, race, and age.

Table 4. Risks of advanced colorectal adenoma associated with EPHX1 genotypes and cigarette smoking in the PLCO Cancer Screening Trial

	No tobacco	Long-term quitters (quit ≥10 years)	Current or recent smokers (quit <10 years)		
			All	Light (≤20 cigarettes/d)	Heavy (>20 cigarettes/d)
	OR (95% CI), case/control (*n*)	OR (95% CI), case/control (*n*)	OR (95% CI), case/control (*n*)	OR (95% CI), case/control (*n*)	OR (95% CI), case/control (*n*)
Combined number of $_{113}$Tyr or $_{139}$Arg alleles					
0-1 (low)	1.0, 84/115	1.3 (0.9-2.0), 89/93	2.2 (1.3-3.6), 56/40	2.6 (1.4-4.8), 34/20	1.7 (0.9-3.4), 22/20
2 (medium)	1.2 (0.8-1.8), 96/166	1.2 (0.8-1.7), 107/132	2.3 (1.4-3.7), 71/48	2.5 (1.4-4.4), 42/27	2.1 (1.1-4.0), 29/21
3-4 (high)	1.3 (0.9-2.0), 80/84	1.4 (0.9-2.2), 76/77	4.7 (2.7-8.3), 71/23	4.1 (2.0-8.3), 35/13	5.6 (2.6-12.1), 36/10
OR (95% CI) for 3-4 versus 0-1 high-activity alleles in smoking subgroup	1.3 (0.9-2.1)	1.1 (0.7-1.6)	2.2 (1.2-4.2)	1.6 (0.7-3.8)	3.3 (1.3-8.7)
P_{trend} in smoking subgroup	0.2	1.0	0.02	0.3	0.02

NOTE: Adjusted for gender, race, and age. Subjects who never used cigarettes but used pipes or cigars were excluded. $P_{interaction} = 0.06$ between the EPHX1 combined genotype (3-4 versus 0-2 high–activity alleles) and smoking status (current or recent smokers versus long-term quitters or nonsmoker).

advanced adenoma. Our study and the study by Cortessis et al. (7) were carried out in early detection programs and focused on the distal (left-sided) colon, within reach of a sigmoidoscope, whereas Ulrich et al. (10) studied adenomas in colonoscopy patients and Tiemersma et al. (15) included sigmoidoscopy and colonoscopy-identified subjects. The studies by Ulrich et al. (10) and Tiemersma et al. (15) were carried out in gastroenterology practices, including subjects with right-sided adenomas. Differences in study subject inclusion patterns in screening programs versus gastroenterology practices and differences in the genetic determinants of left-sided versus right-sided adenoma (28, 29) could have contributed to observed risk differentials. Also, our genotyping approach accounted for a closely associated synonymous nucleotide substitution, $G_{119}A$ (Lys_{119}), which could interfere with the genotyping of $Tyr_{113}His$, causing the heterozygotes to be misclassified as $_{113}$His homozygotes (30), affecting 3% of the subjects in our early assay development (data not shown). It remains unlikely, however, that these methodologic considerations could completely account for the discrepant results across studies.

Overall results from these reports, including our large investigation, favor the hypothesis that high epoxide hydrolase activity is related to increased risk of colon tumors among cigarette smokers, although further investigation is needed to fully assess this result. High predicted activity of EPHX1 also has been associated in some studies with increased risk of lung cancer (31) and other malignancies (32-34), suggesting that these gene variants may have a broader role in human carcinogenesis.

In our data, meat and dietary PAH intake did not modify adenoma risk associated with EPHX1 genotypes. Cortessis et al. (7) found that high predicted activity of EPHX1 was associated with increased adenoma risk in individuals who regularly ate well-done meat, whereas Ulrich et al. (10) reported elevated adenoma risks associated with low predicted activity in those with high intake of cooked meat. Diet-EPHX1 relationships are more difficult to interpret than smoking-EPHX1 relationships; reporting on diet is less accurate (10) and PAH doses in diet are typically lower and more poorly correlated with PAH biomarkers (9, 35).

EPHX1 has a broad tissue distribution and substrate specificity (36). The coding region substitutions we studied appear to influence enzymatic activity through alteration of protein stability (11) rather than enzyme kinetics (11, 36), and may account for only a fraction of all variation in human EPHX1 activity (37, 38). Additional genetic variants, possibly in regulatory regions of the EPHX1 gene, and variation in transcriptional and posttranscriptional modification may also play a role (36, 37). Investigation of other polymorphic variants in EPHX1 (38, 39), particularly those in the 5′-flanking promoter region, the broader evaluation of haplotypes in the gene region, and further evaluation of gene-variant functional significance may provide a more complete picture of EPHX1-associated risks for colorectal tumors.

The conversion of benzo(*a*)pyrene to reactive products depends on a cascade of biotransformations, including oxidation by CYP1A1 to the active benzo(*a*)pyrene 7,8-epoxide, hydration by EPHX1 to the less toxic benzo (*a*pyren 7,8 diol, followed by oxidation through a *P*450-dependent mechanism to the highly carcinogenic BPDE, detoxification of benzo(*a*)pyrene and BPDE by glutathione *S*-transferases, and detoxification by NQO1 of quinones derived from benzo(*a*) pyrene 7,8 diol through aldo-keto reductase (12, 40). We are expanding our investigations to evaluate tobacco use–associated risks involving polymorphic variants in these genes (41, 42)

In summary, our analysis shows that two nonsynonymous microsomal epoxide hydrolase variants ($_{113}$Tyr and $_{139}$Arg) are associated with increased risk of advanced colorectal adenoma, particularly among current and recent smokers, implicating this gene and PAHs in tobacco-associated colorectal carcinogenesis.

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